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Transaminase Enzyme Activity and Histopathology Evaluation of Rat's Liver Induced by DMBA with Temulawak Extract (*Curcuma xanthorrhiza*)

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***Abstract*— Temulawak rhizome (*Curcuma xanthorrhiza*) contains curcumin and xanthorrhizol, which may be used as breast cancer drugs and hepatoprotection. This study aims to analyze the activity of alanine and aspartate transaminase enzymes and the histopathological picture of 7,12-Dimethylbenz(α)anthracene (DMBA)-induced rat liver due to the administration of temulawak extract. This study used 28 female white rats, divided into seven treatment groups, a control group (normal and DMBA) and a treatment group induced by temulawak extract orally with doses of 35, 70, 140, 210, and 280 mg/kg Body Weight (BW) during the 11-week study period. The results obtained were that the application of temulawak extract had a significant effect on alanine transaminase (ALT) levels but did not differ between groups. Aspartate transaminase (AST) levels differed at a 35 mg/kg BW dose. Temulawak extract at a dose of 140 mg/kg BW, the *De Ritis* ratio at normal values. The results of the histopathological analysis showed hepatocyte repair and sinusoidal dilatation were less than optimal at a dose of 140 mg/kg BW. This study concluded that the temulawak extract showed a significant but insignificant difference in the ALT value. The AST value significantly differed in administering temulawak extract at a dose of 35 mg/kg BW. The dose of 140 mg/kg BW controls the value of the *De Ritis* ratio to the normal value. Temulawak extract is expected to improve the healing of the liver damaged by carcinogenic substances.**

***Keywords*— Breast cancer; curcumin; DMBA; hepatoprotection; xanthorrhizol.**

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I. INTRODUCTION

Cancer (carcinoma) is a complex disease that occurs due to fundamental changes in the biological function of cells so that they become independent in growth signals, are insensitive to anti-growth signals, are resistant to apoptosis, have an effect on DNA repair, have unlimited replication potential, angiogenesis, invasion, and metastasis to other tissues [1]. Breast cancer ranks first in new cases and deaths from cancer [2]. This cancer has the highest number of new cases, 2,261,419 (11.7%) [3].

Several risk factors for breast cancer have been reported. The most common risk factors are advancing age and the female sex. BRCA 1 and 2 gene mutation accounts for about 10% of breast cancers. History of ductal carcinoma, high body mass index (BMI), first birth at age greater than 30 years or nulliparity, early menarche (before age 13 years), family history of breast or ovarian cancer, late menopause, and postmenopausal hormone therapy use are the other known risk factors [4].

The development of tumor pathogenesis in the breast gland usually begins with abnormal proliferation that remains confined to its original location, not invading other locations like surrounding normal tissue or other distant body sites. A Benign tumor can then develop into a malignant tumor with metastatic capability. The term cancer is only used in malignant tumors [5]. More malignant breast tumors will make the treatment more difficult. Their subtypes influence the treatment method of breast cancer patients. Breast cancer subtypes are divided into four types include luminal A, and luminal B (which can be human epidermal growth factor receptor 2 [HER2]-positive and HER2-negative), HER2-positive, and triple-negative cancer [6].

Many anticancer drugs have limited selectivity because they must target sufficient therapeutic agents to parts of cells with a high proliferation rate so that the effectiveness of these drugs can be achieved without affecting healthy tissue [7].

Cancer treatment is currently still focused on synthetic drugs, and the drug is a specialist with a narrow therapeutic index that can cause severe toxic effects on the body. Drugs with very narrow therapeutic windows difficult to render an effective therapeutic dose without experiencing toxic effect [8].

One of the medicinal plants that have been shown to have potential in the treatment of cancer is *temulawak*. *Temulawak rhizomes contain xanthorrhizol* which has antimicrobial, *anti-inflammatory, antioxidant, nephroprotective, antitumor, anticancer*, and *hepatoprotective* activities [9]. Research by Ismail et al. [10] showed that the CH_2 group or OH group of the β-di ketone and phenolic OH groups play an important role in the biological activity of *curcumin*. The combination of *xanthorrhizol* and *curcumin* has good activity as an antiproliferative agent for MDA-MB-231 breast cancer cells [11]. However, there has been no toxicity test on Sprague Dawley rats' liver in treating breast cancer by subcutaneously injected DMBA in the mammary glands.

DMBA compounds can initiate mutations in a gene that can cause breast cancer [12]. DMBA metabolism in the liver can interfere with other metabolic processes in the liver even though that organ is not the target of carcinogenesis. DMBA metabolites, such as proximate and ultimate, are formed in liver cells, which are then transported to the mammary glands to produce DMBA-DNA adducts. Analysis of DMBA metabolism in the liver can provide information about the systemic effects of the metabolism of these compounds so that they can describe the activity of DMBA, which can cause breast cancer [13].

Several factors, including the type of chemical substance, large dose, and duration of exposure to the substance, influence liver damage due to toxic substances. The toxic response will increase as the concentration of a given compound increases. Stages of liver damage begin with the onset of hepatocyte necrosis to liver failure [14]. The enzymes AST and ALT are commonly used as screening tests or examination of liver cell damage (hepatocellular injury) which can be caused by various etiologies [15]. Drugs can reduce decreased liver function due to the administration of toxic substances with hepatoprotective properties. The hepatoprotective drugs can stimulate hepatic function, protect, and help regenerate the hepatic cells [16].

This study aims to analyze the activity of transaminase enzymes and histopathological features of the liver in female Sprague Dawley rats induced by DMBA and the administration of *temulawak* extract. *Temulawak* extract in this study is expected to have an effect as a hepatoprotection in treating breast cancer. This research is expected to provide scientific information on the effect of *temulawak* extract on liver function in treating breast tumors through DMBA induction. The information obtained is expected to be a consideration in developing temulawak extract as a breast tumor drug.

II. Materials and Method

A. Reagents and Materials.

The materials used in this study were female white rats of the Sprague Dawley strain, *temulawak* extract standardized by PT Phytochemindo Reksa (Bogor, Indonesia), 7,12-dimethylbenz(a)anthracene (DMBA) (Sigma-Aldrich Co, Missouri). , USA) in olive oil, ketamine Ket-A-100 (Agrovet, Lima, Peru), xylazine Xyla 2% (Interchemie, Waalre, Netherlands), AST and ALT commercial kit (Reiged Diagnostic, Jakarta, Indonesia), carboxymethyl cellulose (CMC) 1%, formalin neutral buffer solution (BNF) 10%, absolute alcohol, xylol, and hematoxylin-eosin dye.

B. Flow Chart

Fig. 1 shows the flow chart of the research. This research has obtained approval for ethical treatment from the Animal Ethics Commission of the Institute for Research and Community Service (LPPM) IPB University with number 174-2020 IPB. All the method was done according to the ethical treatment suggestion. This research aims to provide the activity of transaminase enzyme and histopathology evaluation related to the effect of temulawak extract on liver function in treating breast tumors through DMBA induction.

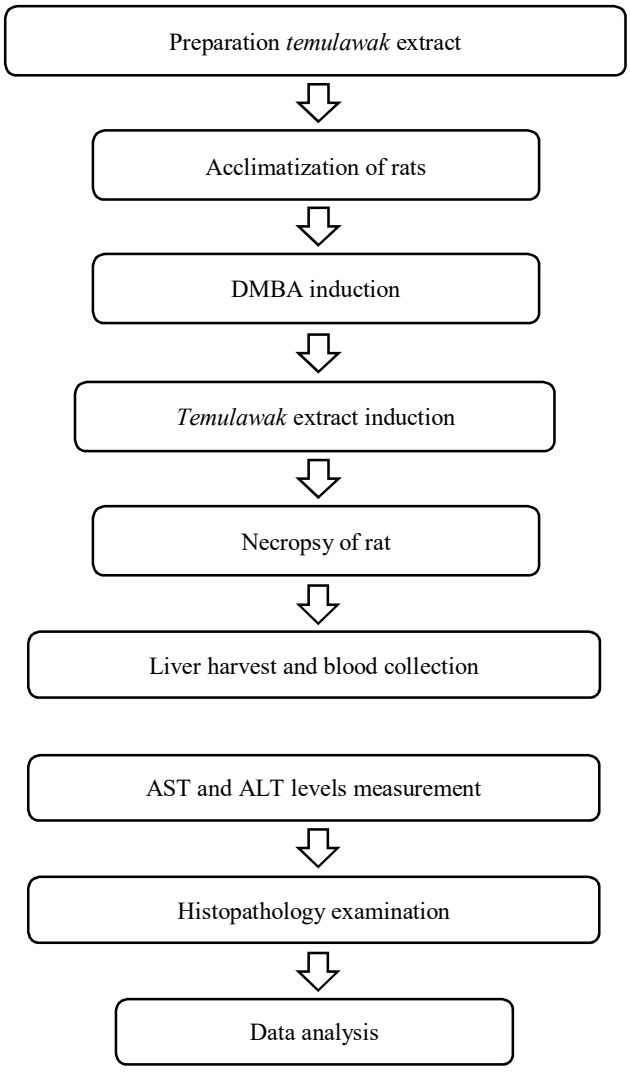

Fig. 1 Flow chart of the research's method

C. Sample Preparation

Standardized *temulawak* extract was obtained from PT Phytochemindo Reksa (Bogor, Indonesia). *Temulawak* extract was made in 5 doses of 35, 70, 140, 210, and 280 mg/kg BW. *Temulawak* extract was weighed, and 1% CMC powder was added, then dissolved in distilled water. The

finished extract is then stored in a glass bottle for easy storage. Storage is carried out at a temperature of 2 - 4 °C in a refrigerator.

D. DMBA dilution

DMBA powder was weighed on an analytical balance in a fume hood regarding the carcinogenic properties of the material. The dose of DMBA was 20 mg/kg rat weight. The results of the scales are then dissolved in olive oil. The diluted DMBA solution was then stored in a glass bottle for easy storage. Storage is carried out at a temperature of 2 - 4 °C in a refrigerator.

E. Experimental Animal Conditioning

This study used 28 female Sprague Dawley rats. All rats were acclimatized in the experimental cage for two weeks with the aim of uniform health conditions, nutrition, and environmental conditions. During this time, the rats were provided with commercial feed and water ad libitum. Rat bedding is made from coarse sawdust with replacement 2 times a week. Room lighting is obtained naturally through windows at room temperature. The cage is also equipped with an exhaust fan to maintain airflow and dissipate excess heat.

F. DMBA Induction

DMBA solution in olive oil at a dose of 20 mg/kg. The BW of the rats to be induced was weighed first. The injection was done subcutaneously into the third mammary from below on the left side of the rat's body. DMBA injections were performed two times, and the induction process had a span of 1 week from the first injection.

G. Treatment of Rats

A total of 28 rats were divided into 7 groups consisting of 5 treatment dose groups, 1 DMBA control group, and 1 normal control group each with 4 mice. The normal group (NO) was the control group that was not given any treatment. The DMBA group was a control group induced by DMBA without giving *temulawak* extract. The treatment group that was given *temulawak* extract and DMBA was divided into 5 groups with various doses, including: (1) Group one (K1) was given *temulawak* extract at a dose of 35 mg/kg BW; (2) Group two (K2) was given *temulawak* extract at a dose of 70 mg/kg BW; (3) Group three (K3) was given *temulawak* extract at a dose of 140 mg/kg BW; (4) Group four (K4) was given *temulawak* extract at a dose of 210 mg/kg BW; (5) Group five (K5) was given *temulawak* extract at a dose of 280 mg/kg BW;

After the acclimatization process for 2 weeks was completed, it was continued with the DMBA induction procedure. DMBA treatment and control group induced DMBA through subcutaneous injection of mammary tissue. *Temulawak* extract was administered orally every 3 days for 11 weeks, i.e., 5 weeks after the second DMBA induction. All groups of mice were necropsied at week 11 after treatment.

H. Termination of Test Animals

The rat termination process was carried out at week 11 after treatment. Rats were anaesthetized with a solution of ketamine and xylazine via the intraperitoneal injection route at a dose of 10 mg/kg BW and 1 mg/kg BW, respectively, followed by blood collection and necropsy. Blood was taken via the intracardial route and then put into a 5 mL Vaculab Plain blood tube and continued with the centrifugation process at 3000 rpm for 15 min.

I. Measurement of the activity of ALT and AST enzymes

Blood samples obtained from the heart of rats were centrifuged at 3000 rpm for 15 min to obtain blood serum. Blood serum was then measured for ALT and AST activity with a commercial kit according to the 1986 International Federation of Clinical Chemistry (IFCC) method. 50 L of blood serum sample was added with 500 L of the reagent mixture. The mixture was then homogenized, incubated at 37 °C for 60 seconds, and read the absorbance. The incubation was then carried out again, and the absorbance was read at the 1st, 2nd, and 3rd min of incubation. Absorbance readings were carried out using a BMG Labtech SPECTROstar Nano spectrophotometer (Ortenberg, Germany) with a wavelength of 340 nm. The following formula then calculates the absorbance results obtained

$$\text{ALT (U/L)} = \text{Abs/min} \times 1768 \times 2 \quad (1)$$

$$\text{AST (U/L)} = \text{Abs/min} \times 1746 \times 2 \quad (2)$$

J. Liver Sampling

The rats were necropsied, and the liver was removed and then put into a container containing 10% neutral formalin (BNF) buffer solution for at least 3 x 24 hours.

K. Making Histopathological Preparations

The rat liver that had been fixed in 10% BNF solution was then cut into thin tissue and put into a tissue cassette. The dehydration process follows the next stage. The dehydration process was carried out by immersion in graded alcohol (ethanol 70%, 80%, 90%, 96%, absolute I, and absolute II). The remaining ethanol was clarified with xylol I, II, and III. This step was followed by infiltration in liquid paraffin at 60 °C for 30 min 4 times. The mold that will be used before is washed with a mixture of 96% ethanol, xylol, and distilled water. Hot paraffin is poured into the mould block until it reaches half the volume of the mould. Then the embedding stage is carried out, where the tissue is carefully implanted into a mold containing liquid paraffin. This process must be carried out carefully so that the tissue does not touch the bottom of the mold, which is then covered again with liquid paraffin. The paraffin-hardened tissue was cut with a microtome of 4-5 m thickness. The results of the pieces are put into warm water at 40 °C, aiming to melt the paraffin so that the folds of the pieces can be lost. The preparation was placed on a slide and dried in an incubator at 60 °C for one night.

L. Hematoxylin-Eosin staining

This process begins with deparaffination using xylol I and II for 2 min each and rehydrated with alcohol in stages (deparaffinization-rehydration). Tissue sections were rehydrated with absolute alcohol for 2 min then with 95% and 80% ethanol for 1 minute respectively. The preparation was then rinsed with running water for 2 min. The next stage was followed by tissue preparation stained with hematoxylin (Mayer's dye) for a span of 8 min which was then rinsed with running water. The stained tissue was then put into a lithium

carbonate (LiCO) solution for 30 seconds and again rinsed with running water for 30 seconds. Next, the tissue preparations were stained with eosin dye for 2-3 min and rinsed with running water for 1 minute.

The process was then continued with tissue preparations which were re-dehydrated with graded alcohol, namely 95% ethanol and absolute ethanol I for 10 dips of each liquid, and then alternated with absolute ethanol II for 2 min. The preparation was then clarified using xylol I for 1 minute and continued with xylol II for 2 min. The results of the HE stains were then applied with Entellan adhesive and then covered with a coverslip. The preparations were dried and observed with a light microscope (Olympus BX 51) equipped with a camera. Each preparation was viewed as many as 5 fields of view with a magnification of 20x and observed patterns of arrangement and shape of liver cells.

M. Data Analysis

This study was designed using a completely randomized design (CRD). Statistical analysis of AST and ALT enzyme activity was performed using the ANOVA method at a 95% confidence interval. A qualitative assessment of the condition of liver tissue was carried out on the results of histopathological observations of the liver.

III. Results and Discussion

A. Blood Serum Transaminase Enzyme Activity

Measurement of transaminase enzyme activity by testing blood serum ALT and AST. The rat blood serum was taken on the 75th day after extract induction. The activity of the ALT enzyme is shown in Figure 2. This test resulted in the normal group ALT enzyme activity of 170.435 U/L. The group that had the highest ALT activity value was in the K2 group, namely 189,530 U/L, with a value that was not significantly different (P>0.05) from the normal group. The measurement data results showed that the values decreased in the K1 and K3 groups and then increased again in K4 and K5. The ALT value that is closest to the normal value is in K5.1.

The activity of the AST enzyme is shown in Fig 3. The normal group AST value was 15,811 U/L. The K1 group had the highest AST value, which was 121,929 U/L. the value closest to the normal group value is in group 5 of 19,148 U/L. Overall, the AST values obtained were above the normal group values. AST levels in the treatment and normal groups were not significantly different (P>0.05).

The AST/ALT ratio, also known as the *De Ritis* ratio, is used to indicate the level of liver damage. The normal value of the *De Ritis* ratio is around 0.8 [17]. A value of <0.8 indicates that there is mild damage that is acute, while chronic damage causes the value of the *De Ritis* ratio to be > 0.8 [18]. According to Table 1, the highest *De Ritis* ratio was in the treatment group 1, with a dose of 35 mg/kg BW of 1,302 being an indicator of chronic damage. Group 3 with a *De Ritis* ratio of 0.866 can be classified as having a normal liver condition. The other treatment groups, the normal group, and the DMBA group had *De Ritis* values below 0.8, indicating acute damage.

The data that can be seen in Figure 2 and Figure 3 shows that the overall ALT value has a high value, and the AST value has a low average value. The high ALT value may be related to the use of ketamine-xylazine anesthetics [19], the use of ketamine-xylazine anesthesia can increase ALT values which will last for 24 hours. In contrast to the ALT value, the mean AST value obtained in this study was below the normal range value. This low level of AST may be related to feeding that does not contain enough protein or amino acids needed for the formation of these enzymes so that the production of these enzymes is low [20].

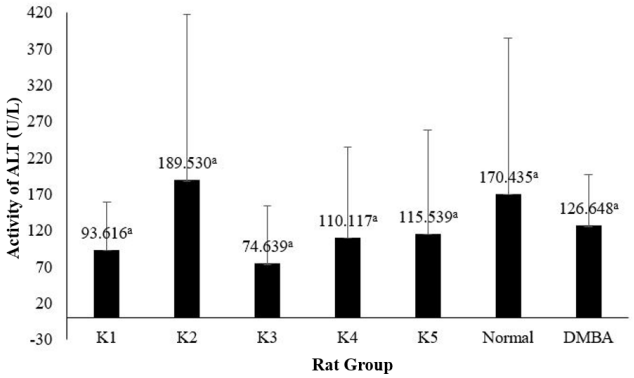

Fig. 2 The activity of ALT enzymes in each group

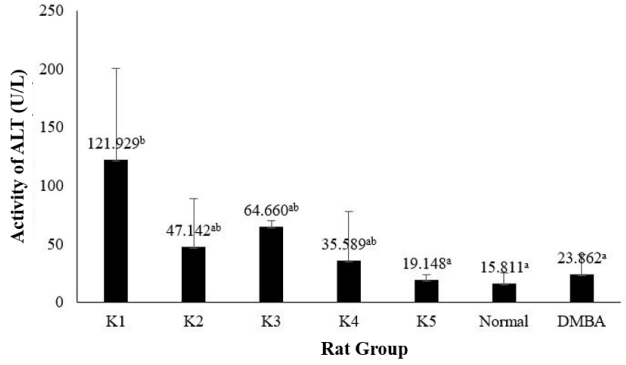

Fig. 3 AST enzyme activity in each group

TABLE I
VALUE OF DE RITIS RATIO (AST/ALT RATIO)

Group	Temulawak Extract Dosage	AST/ALT
1	35 mg/kg BW	1.302
2	70 mg/kg BW	0.249
3	140 mg/kg BW	0.866
4	210 mg/kg BW	0.323
5	280 mg/kg BW	0.166
Negative control	-	0.093
DMBA control	-	0.188

The value of ALT levels presented in Figure 2, shows a value that tends to decrease in groups 1 and 3 and then increases again in groups 4 and 5. The increase in ALT values in groups 4 and 5 is due to the larger the dose of the extract, the amount of the drugs entering the body will increased [21]. At certain levels, the high content of phytochemicals in the extract can cause toxic effects, which are responded to by the liver. Group 2 (*temulawak* extract dose of 70 mg/kg BW) and the normal group had very high scores. This is possible due to blood lysis. According to reports in Paul [22], blood lysis will cause the release of ALT from erythrocytes so that an increase in ALT levels can occur.

This overall decrease in ALT levels indicates a good synergism of the content of the *temulawak* extract. The content of *temulawak* consists of *curcumin* and *xanthorrhizol* [23]. Based on reports by Cheah et al. [11] and Fitria et al. [24] stated that curcumin and *xanthorrhizol* compounds can suppress the growth of breast cancer accompanied by a hepatoprotective effect. The AST enzyme activity values in the treatment group and DMBA group had values that were above the normal group values. This is due to the DMBA induction reported by Dosumu et al [25] can increase AST levels. Researchers [26] also reported that the AST enzyme will be more produced when breast tumor growth occurs. Increasing the dose of *temulawak* extract showed a decrease in AST enzyme activity. This decrease in AST enzyme activity indicates an improvement in the liver provided by the extract. Group 1 given the *temulawak* extract 35 mg/kg BW had the highest AST enzyme activity with a level of 121,929 U/L which indicated that this dose was still unable to provide a good hepatoprotective effect where the hepatoprotective effect of *temulawak* according to Ardiyanto et al [27] observed at 100 mg/kg BW.

Through the AST and ALT data that have been obtained, conclusions can be drawn through the calculation of the *De Ritis* ratio. By the Chalmers et al. [28] revealed that group 1 is suspected of having chronic damage while the other group indicates acute damage to the liver. Calculations showed that the temulawak extract at a dose of 140 mg/kg BW in group 3 had a De Ritis ratio at a normal value. The value of the *De Ritis* ratio of group 3 of 0.866 has a normal value [18]. This indicates a process of reducing the toxicity effect of DMBA as a xenobiotic so that it does not cause damage to the liver.

DMBA as a carcinogenic compound is known to be potent in the formation of cancer in animal models. DMBA is a xenobiotic substance that will be absorbed by the body and carried by the blood to the liver as a place of metabolism after being injected. Although the liver is not the target organ of DMBA carcinogenesis, the process of formation of DMBA metabolites occurs in the liver. These metabolites can then move to the mammary glands and form DNA adducts (DNA that binds to carcinogenic compounds). DMBA metabolism in the liver involves several stages involving phase I and II enzymes [25].

DMBA is classified as a compound whose carcinogenesis mechanism is an indirect-acting carcinogen or a procarcinogen that requires metabolic activation. This process involves phase I enzymes and epoxide hydrolase. Phase I enzymes consist of cytochrome p-450 (CYP) system enzymes, for example, CYP1A1 and CYP1B1. These enzymes are important in converting carcinogenic chemical compounds such as DMBA into electrophilic and non-electrophilic compounds [11]. This process changes the form of DMBA from its inactive form to its active form. The inhibition of cytochrome enzyme activity will reduce the formation of proximate and ultimate carcinogens, decreasing the risk of carcinogenesis [29].

Several pathways can be used in DMBA metabolism, one of which is the formation of electrophilic epoxide hydrodiol reactive intermediates. DMBA metabolism in *carcinogenesis* begins with the oxidation of double bonds by CYP enzymes, forming DMBA-3,4-epoxide. This step is followed by the microsomal epoxide hydrolase (mEH) enzyme, which hydrolyzes the epoxide to DMBA-3,4-dihydrodiol. DMBA-3,4-dihydrodiol is a proximate carcinogen metabolite. The enzymes CYP1A1 or CYP1B1 then oxidize DMBA-3,4-dihydrodiol to form the ultimate carcinogen DMBA-1,2-epoxide-3,4-dihydrodiol (DMBA-DE) [30].

The presence of DMBA induces the formation of enzymes CYP1A1 and CYP1B1 which the liver and mammary glands can express. The process of forming enzymes CYP1A1 and CYP1B1 is mediated by the cytosolic aryl hydrocarbon receptor (AHR). AHR that binds to Polycyclic aromatic hydrocarbon (PAH) compounds then translocate to the nucleus. The process is continued by heterodimerization with the AHR nuclear translocator (ARNT) and triggers transcription of the CYP1A1 and CYP1B1 genes [30].

Purine nitrogen bases adenine (A) and guanine (G) are highly susceptible to PAH group carcinogens. The *dihydrodiol* epoxide formed will bind covalently to the exocyclic amino group of deoxyadenosines (dA) or *deoxyguanine* (dG). This reaction will produce a stable DNA adduct bond so that it can initiate cancer formation [31].

The formation of DNA adducts is involved in the process of tumorigenesis through several molecular or genetic changes such as DNA strand breaks, chromosomal damage, or mutations [31]. DNA adducts are *premutagenic,* which can change the basic coding properties of DNA and can cause mismatches during DNA replication. Mutations that occur in important genes (e.g., oncogenes, tumor suppressor genes, or cell cycle control genes) can initiate cells that have proliferative and survival capabilities beyond normal cells. These mutated cells (cancer cells) do not have the growth control to perform cell cycle arrest. The addition of cancer cell mass will cause tumor development [32].

DNA damage by DMBA will cause the activation of complex signaling pathways as a genomic defense. The protein that has the main function as the one responsible for DNA damage is the p53 protein. The p53 protein as a tumor suppressor will lose its function and cause tumor formation. This is followed by the accumulation of p53 in the nucleus and is activated after phosphorylation. Active p53 regulates many downstream signals, such as DNA repair, cell cycle inhibition, and apoptosis. Increased expression (upregulation) of p53 can activate pro-apoptotic genes that will cause mitochondrial dysfunction and trigger the release of cytochrome c, which stimulates mitochondrial apoptosis [33].

The body's defense against damage caused by carcinogenic compounds is carried out by phase II enzymes such as glutathione S-transferase (GST) and UDP glucuronyltransferase (UDP-GT). The mechanism is conjugation with glutathione/glucuronic acid so that electrophilic and non-electrophilic metabolites can be removed. This conjugation also forms detoxification products whose reactivity decreases and is more water-soluble (increased polarity), making it easier to be excreted in the bile and in the urine [33].

According to Shahcheraghi et al. [34] the curcumin content of *temulawak* (*Curcuma xanthorrhiza*) can activate phase II antioxidants levels in a several ways, like activating BALF, hypoxia-inducible factor 1(HIF-1α), and inhibiting vascular endothelial growth factor (VEGF). This indicates that there is potential for temulawak in increasing the biotransformation of xenobiotic compounds to be excreted out of the body.

Carcinogen compounds as xenobiotic compounds can be increased their detoxification process through this mechanism. In addition, curcumin also affects decreased hepatocellular fat storage, improves liver function profile, and boosted the metabolic function of CYP3A and CYP7A [34]. Through culture and animal studies, it was also reported that curcumin may have the ability to inhibit the activity of cytochrome p450 (CYP) enzymes such as CYP1A1 [35]. Curcumin also reported induced cell death in a dose-dependent manner. Typical apoptotic markers showed by [36], such as DNA fragmentation, phosphatidylserine exposure, and caspase cleavage.

Xanthorrhizol contained in *temulawak* also shows great potential in reducing tumor malignancy by inducing breast cancer cell death through modulation of Bcl-2, p53, and PARP-1 accompanied by organ protection effects and antimetastatic activity [11]. Hepatotoxicity-reducing activity has also been reported to be carried out by *xanthorizol*. A possible pathway is decreased phosphorylation of c-Jun N-terminal kinases (JNK) [37]. *Xanthorrhizol* or *curcumin* also can regulate DNA binding to the transcription factors nuclear factors kappa B (NF-αB) and AP-1 [38].

According to Cheah et al [11], the combination of *xanthorrhizol* and curcumin can induce apoptotic cell death as indicated by changes in membrane potential, DNA condensation, cell shrinkage, and DNA fragmentation. This is accompanied by other positive effects, namely antimetastatic activity, the protective effect of *xanthorrhizol*, and antiproliferative activity of *curcumin-xanthorrhizol*. Through the reports, treatment with a combination of curcumin and *xanthorrhizol* has a high potential as an antiproliferative agent for the treatment of invasive breast cancer cells. The combination of compounds contained in *temulawak* is also believed to protect the liver as a hepatoprotective [38].

AST and ALT enzymes are commonly used as screening tests or examinations of liver cell damage (hepatocellular injury) which can be caused by various etiologies [15]. ALT or Serum Glutamate Pyruvate Transaminase (SGPT) is an enzyme that plays a role in the catalysis of the reaction to transfer the amino group from glutamate to pyruvate and produce the amino acid alanine. ALT has a role in the transport of amino groups that will be removed through the urea cycle from muscle. Muscles that use amino acids as an energy source collect residual amino groups in the form of alanine, which enters the bloodstream to the liver to enter the urea cycle [39].

AST and ALT levels are closely related to liver damage. ALT levels are markers of acute liver damage. Meanwhile, AST levels are markers of chronic liver damage. 20% of AST is found in the cytoplasm of liver cells and another 80% is in the mitochondria of liver cells [40]. ALT and AST enzymes are associated with liver parenchyma cells. The difference between these two enzymes lies in their location where ALT is more abundantly found in the liver. AST can be found in the liver, heart (heart muscle), skeletal muscle, kidneys, brain, and red blood cells. This makes ALT can be used as a more specific indicator of liver inflammation than AST [39].

The measurement of AST and ALT enzyme activity was carried out using a commercial kit. The measurement mechanism for these two enzymes is the same, namely measuring the oxidation speed of NADH in the kit to form $NAD^{+,}$ which is proportional to the activity of the transaminase enzyme and is indicated by a decrease in absorbance at a wavelength of 340 nm [41]. The AST enzyme catalyzes a transamination process in which the amine group of L-aspartate is transferred to the -carbon group of -ketoglutarate. This reaction produces keto acid analogs of L-aspartic i.e., oxaloacetate and L-glutamate as a result of the amination of -ketoglutarate. Oxaloacetate then undergoes reduction along with the oxidation of NADH to NAD^+ catalyzed by the enzyme malate dehydrogenase (MDH) [42].

On the other hand, the ALT enzyme functions in catalyzing the deamination process of L-alanine with the help of -ketoglutarate to form pyruvate. Like the AST enzyme, pyruvate is reduced along with the oxidation of NADH with the help of the enzyme lactate dehydrogenase (LDH). This quantification reaction of pyruvate and oxaloacetate is an indicator reaction that can be measured by observing the NADH oxidation rate through the absorbance value over time at a wavelength of 340 nm. The higher levels of transaminase enzymes are directly proportional to the increase in the amount of NADH oxidized causing a decrease in absorbance as the reaction progresses [42].

B. Histopathology of the Liver

Microscopic testing also needs to be done to see what happens at the cellular level. The presentation of Figure 4 which was carried out at 40x magnification, shows the difference in hepatocyte histology with the damage indicated by the arrowhead symbol. The normal group (NO) did not show any significant hepatocyte damage with clear hepatocyte shape and boundaries and normal sinusoidal spacing.

Various forms of necrosis and apoptosis were found in each treatment group. The significant difference seen during the observation was the occurrence of sinusoidal dilatation in the DMBA and K1 groups. Microscopic improvement by *temulawak* extract was seen with a reduction in sinusoidal necrosis and dilatation from group 1 to optimal in group 3. The condition of cells and sinusoids from group 4 and group 5 showed sinusoidal dilatation. Fat degeneration was also observed in group 4 and group 5.

The liver is an organ that has the function of maintaining metabolic homeostasis. This function is related to the capacity of the liver to eliminate metabolites from nutrients and xenobiotics. This also causes the liver vulnerable to injury from residual metabolites, and toxic substances, to damage from circulation disorders [43].

The images were taken at 40x magnification which is presented in Figure 4. Seven groups were observed to have different appearances of hepatocytes. The microscopic picture of the normal group (NO) showed no significant damage. The arrangement of hepatocytes in this group has a normal size with clear boundaries between hepatocytes. The sinusoid size of this group also did not show any dilatation or widening of the sinusoids.

The histopathological observations of the DMBA group showed a significant difference from the NO group. Some of the abnormalities that occur include sinusoidal dilatation, enlargement of the cell nucleus, apoptosis, and necrosis. This group also has the condition of hepatocytes with a pale color.

This sinusoidal dilatation (widening) condition can be caused by exposure to toxic substances. The lesion is characterized by defined areas of liver parenchyma with variably expanded sinusoidal lumens filled with blood [44]. This situation is in line with the results of research conducted by Patel and Shah [45], the damage caused by DMBA compounds, one of which is the occurrence of sinusoidal dilatation. Other damages that can occur due to DMBA compounds include necrotic changes, leukocytic infiltration, and vacuolar degeneration [46].

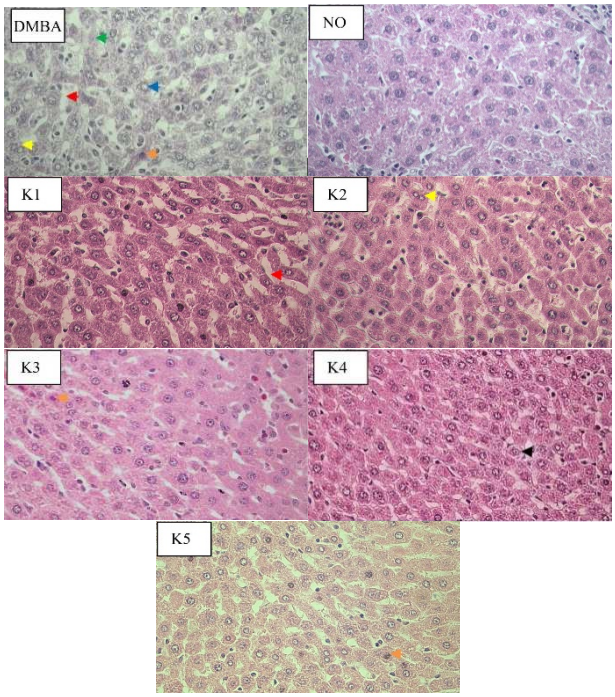

Fig. 4 Liver histopathology with HE stained and 40x magnification. Damage marked symbol (▶): enlargement of the cell nucleus; (▶): sinusoidal dilatation; (▶): necrosis; (▶): apoptosis; (▶): binucleus; (▶): lipid degeneration

Each treatment group had necrotic hepatocytes. Hepatocyte necrosis can be characterized by the occurrence of morphological changes in the cell nucleus, including *pycnotic, karyolitic, and cariorexic*. Pycnotic conditions cause hepatocyte nuclei to shrink to become dense and dark in color due to clumping of chromatin, *karyolitic* is characterized by fading of basophil chromatin, visible cell nuclei are lost, while karyorrhexis is indicated by fragmented cell nuclei becoming small and scattered [47]. Some cells experienced apoptosis in DMBA, K1, K3, and K5 treatments. Apoptosis has morphological changes such as cell shrinkage, chromatin condensation, and formation of apoptotic bodies [48].

This histopathological observation has concordance with the results of ALT enzyme activity. This harmony was shown by increasing the dose of *temulawak* extract which increased histopathological and enzymatic improvement and gave the maximum effect at a dose of 140 mg/kg BW of *temulawak* extract. *Temulawak* extract showed a good hepatoprotective effect on the condition of liver cells [38].

Based on histopathological observation, the liver is composed of hexagonal hepatic lobules. Each lobule is equipped with a central vein surrounded by parenchyma cells (hepatocytes) radially. There is a lot of endoplasmic reticula (ER) in hepatocytes. This organelle functionally works in the process of oxidation, methylation, and conjugation to inactivate or detoxify various substances [49]. Between rows of hepatocytes are lined by sinusoidal endothelial cells that function in the exchange of blood gases, nutrients, and other signaling molecules in and out of the major vessel's portal vein, central vein, and biliary tree [50]. One of the histopathological observations of the liver was performed using the Hematoxylin-Eosin (HE) staining method. This staining is a routine method consisting of hematoxylin as an alkaline dye which gives a blue color to the acidic cell nucleus and eosin as an acidic dye which gives a red color to the alkaline cytoplasm [51].

IV. Conclusion

The results of the analysis showed that the administration of *temulawak* extract showed a significant but not significant difference in the ALT value. The AST value was significantly different when the *temulawak* extract was administered at a dose of 35 mg/kg BW. The dose of 140 mg/kg BW controlled the value of the *De Ritis* ratio to normal values. Histopathological examination also showed an improvement in liver cells and reduced optimal sinusoidal dilatation in the administration of the *temulawak* extract at a dose of 140 mg/kg BW. Giving *temulawak* is expected to improve the work of the liver damaged by carcinogenic substances.

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